
بسم الله الرحمن الرحيم

DNAZYMES

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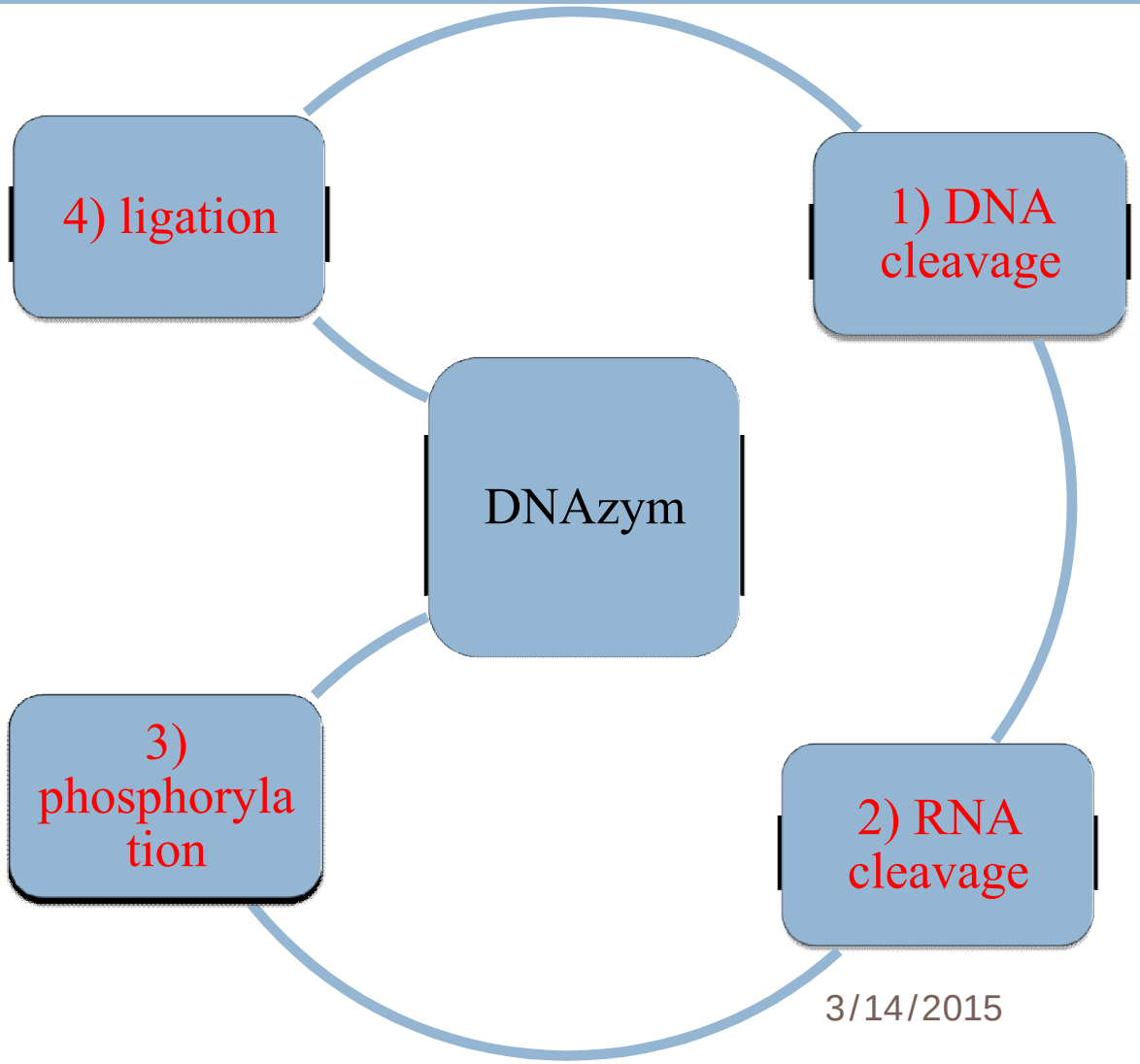
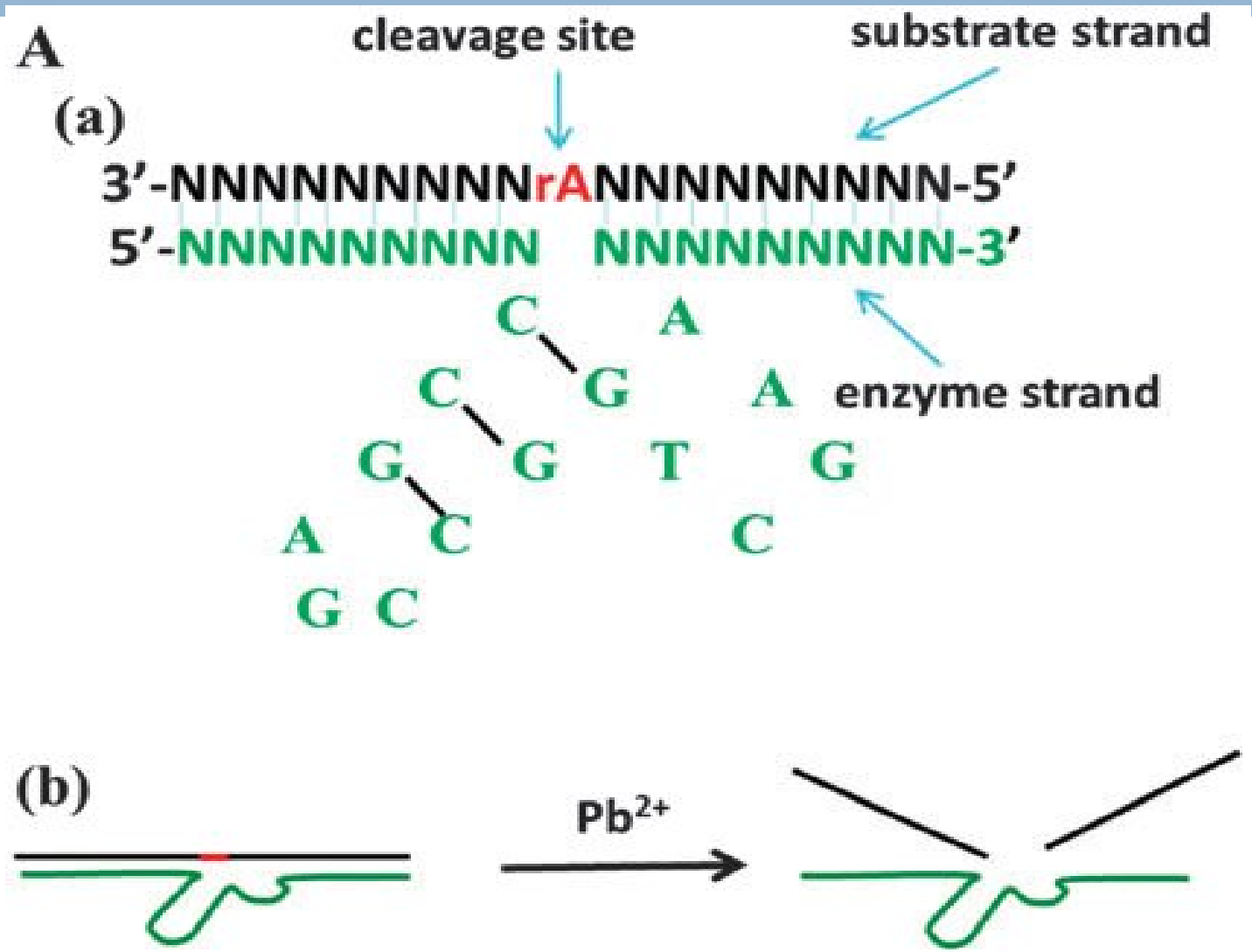
History

- in 1994 by Breaker and Joyce (3)
- trans esterification reaction (3)
- single stranded DNA could indeed act as a catalyst(3)

Process of DNAzym(3)

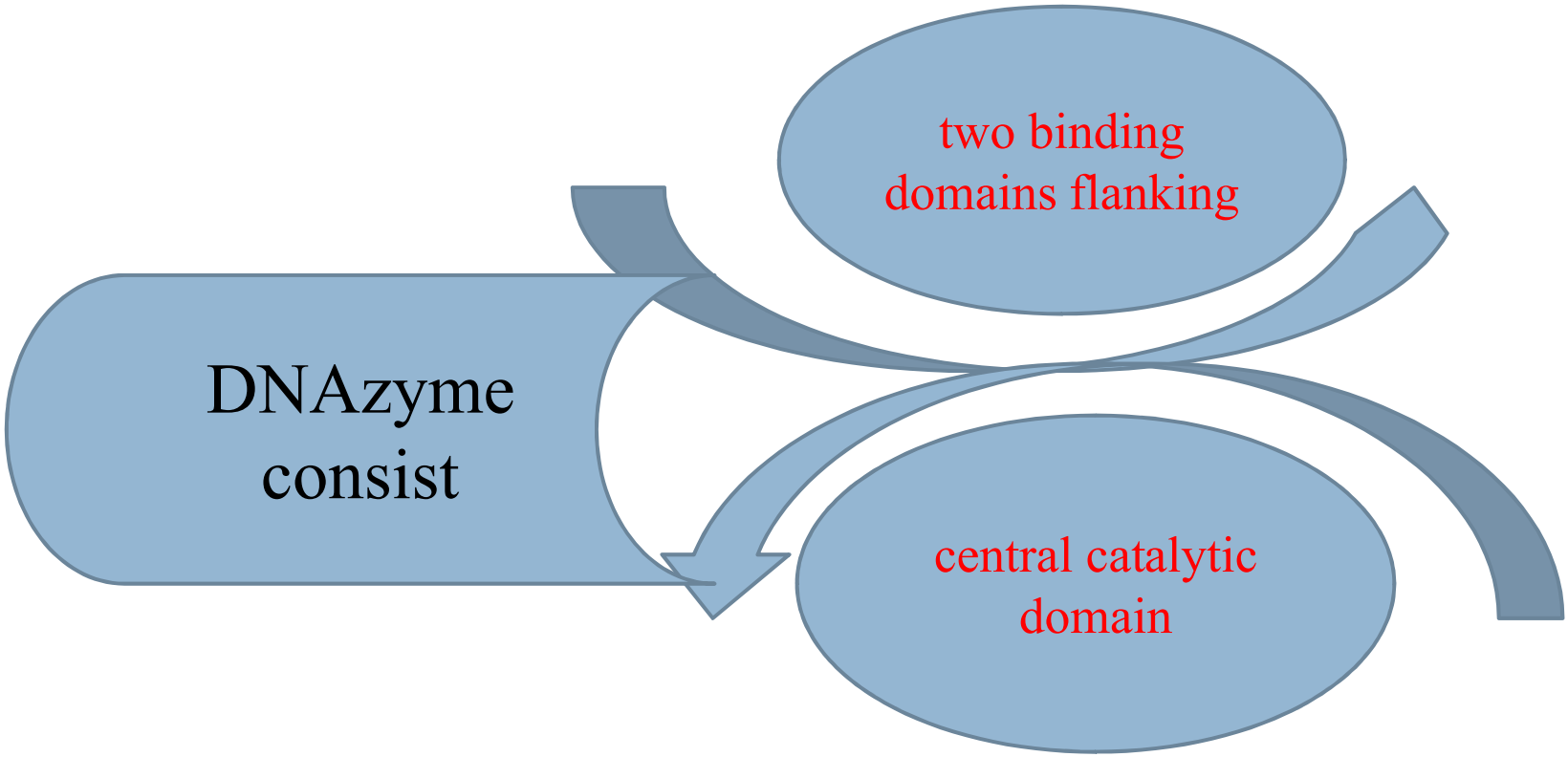



A
cleavage site
substrate strand
(a)
3'-NNNNNNNNNrANNNNNNNNNN-5'
5'-NNNNNNNNNN NNNNNNNNNN-3'
C A
C G A
G G T G
A C C
G C
enzyme strand
(b)
Pb2+

Definition

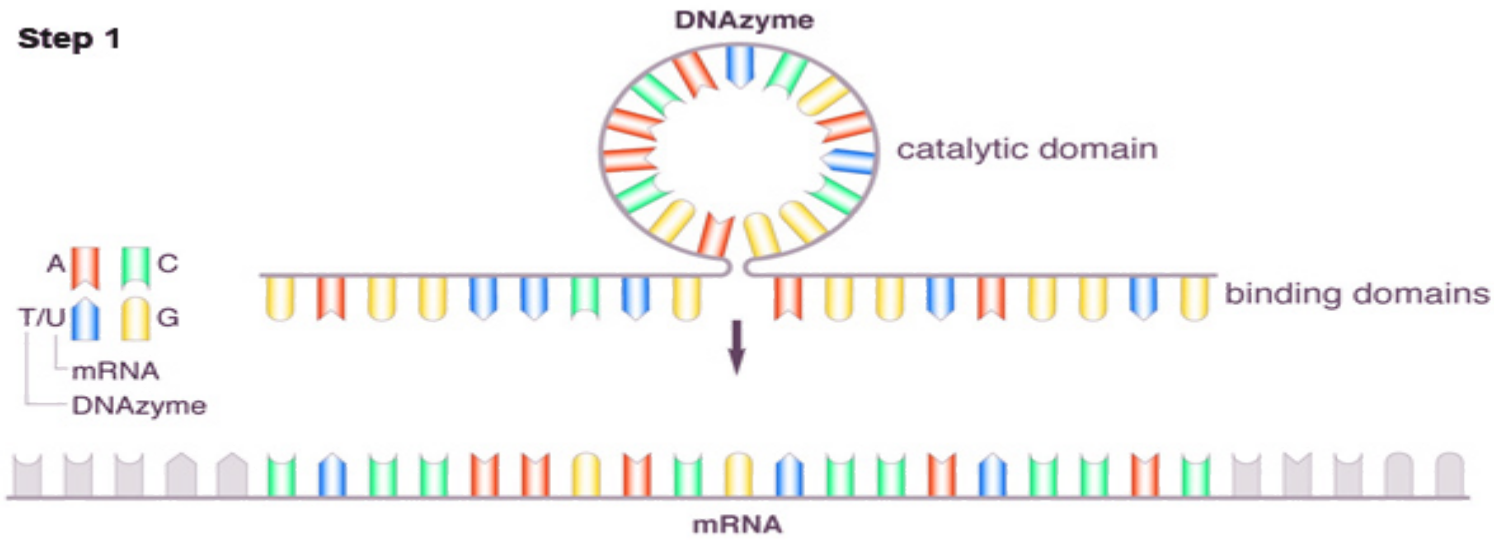
- DNAzymes are catalytically active DNA molecules(6)
- their capability to cleave RNA molecules (6)
- DNAzymes are single-stranded DNA molecules(6)

Structures

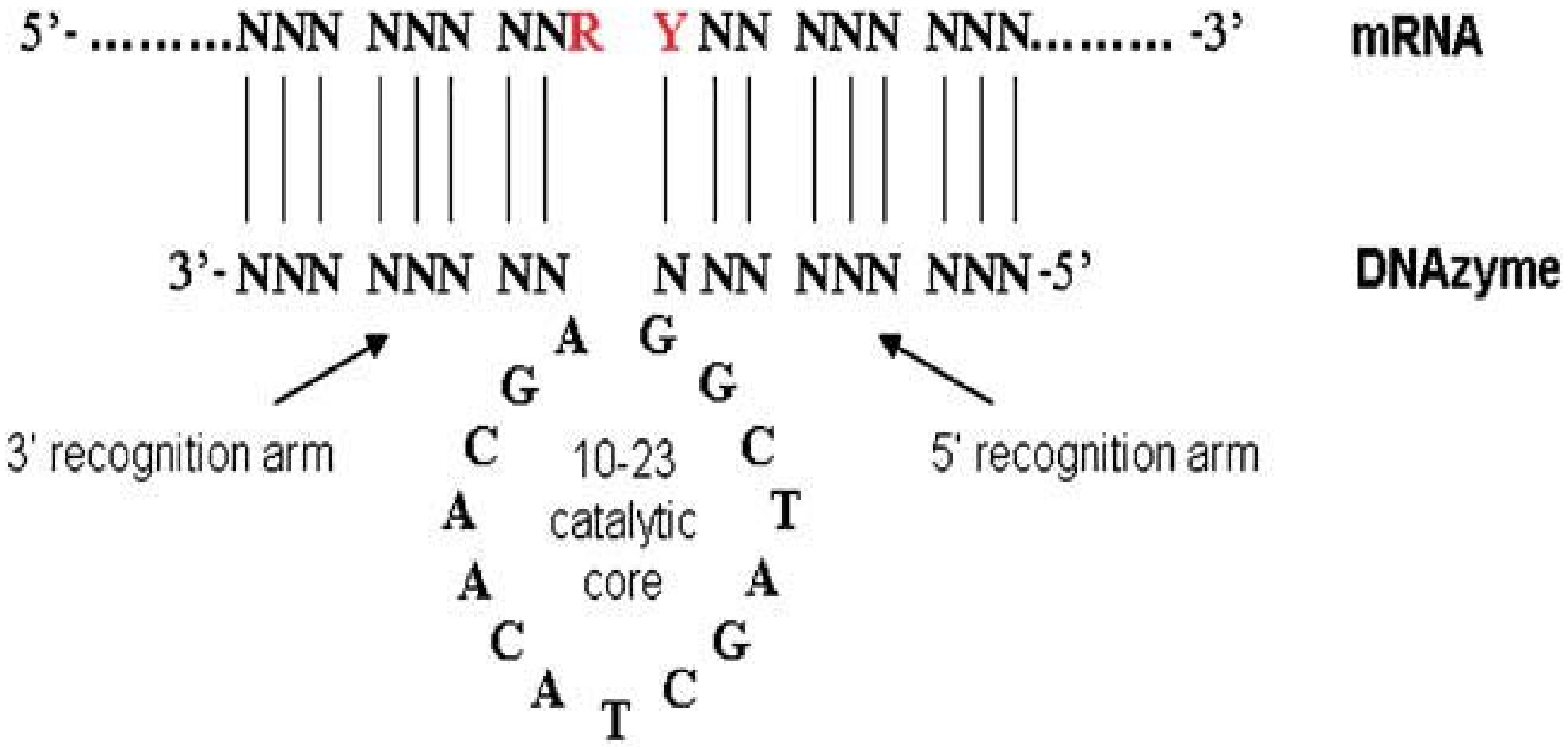

two binding
domains flanking
DNAzyme
consist
central catalytic
domain

- The latter is composed of 15 deoxynucleotide

- the binding domains are variable(9)



Cleaving the mRNA(10)



*The phosphodiester bond between the nucleotides shown in red is cleaved.

- half life from 70 min to 21 h in human serum (2)

- also remain functionally intact for at least 24–48 h in human serum(2)

Characteristic

- DNAzymes represent a particular class of antisense molecule(4)
- inherent catalytic activity which makes them an attractive tools(4)

Main Applications

- also used as active components for constructing nanostructures (3)

- important functional nucleic acids for chemical biology medicine, analytical chemistry and materials science. (3)

Main Applications

- lead to down-regulation of protein expression(5)

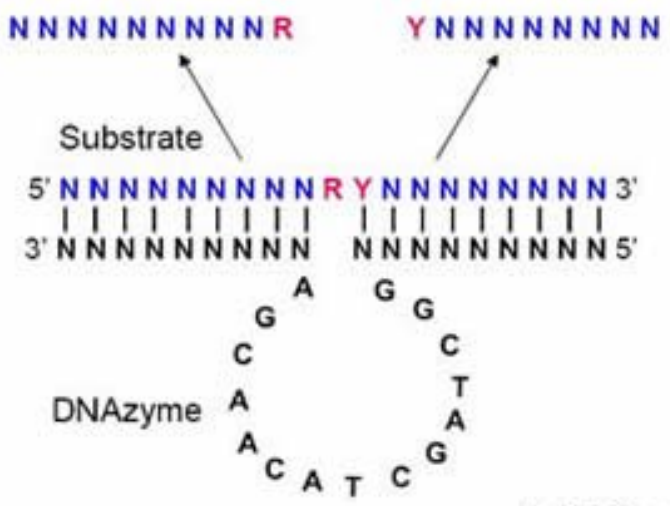
- their use as amplifying labels for the development of optical or electronic sensors(3)

1. In contrast to enzymes that are thermally unstable(8)

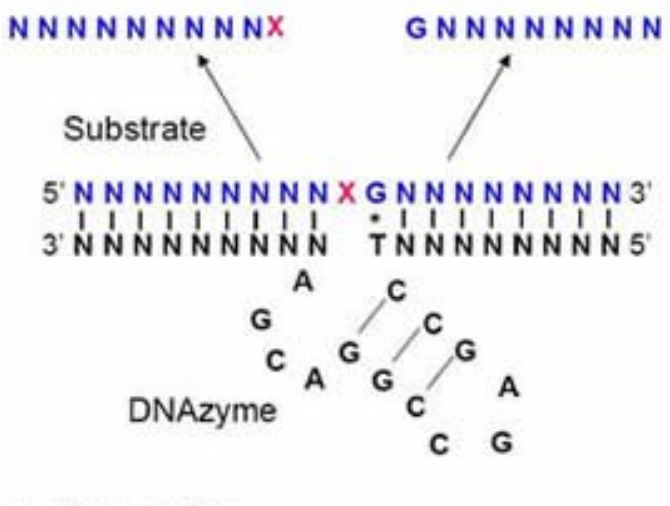
2. impressively stable under ambient and even elevated temperatures(8)

3. may be prepared by the polymerase chain reaction (PCR). (8)

10:23 DNAzyme



8:17 DNAzyme



A, C, T, G, or N (any) = deoxyribonucleotides

R (A or G), Y (U or C) and X (any) = ribonucleotides

N = either deoxyribonucleotides or ribonucleotides

Santoro & Joyce (1997) Proc. Natl. Acad. Sci., USA 94, 4262

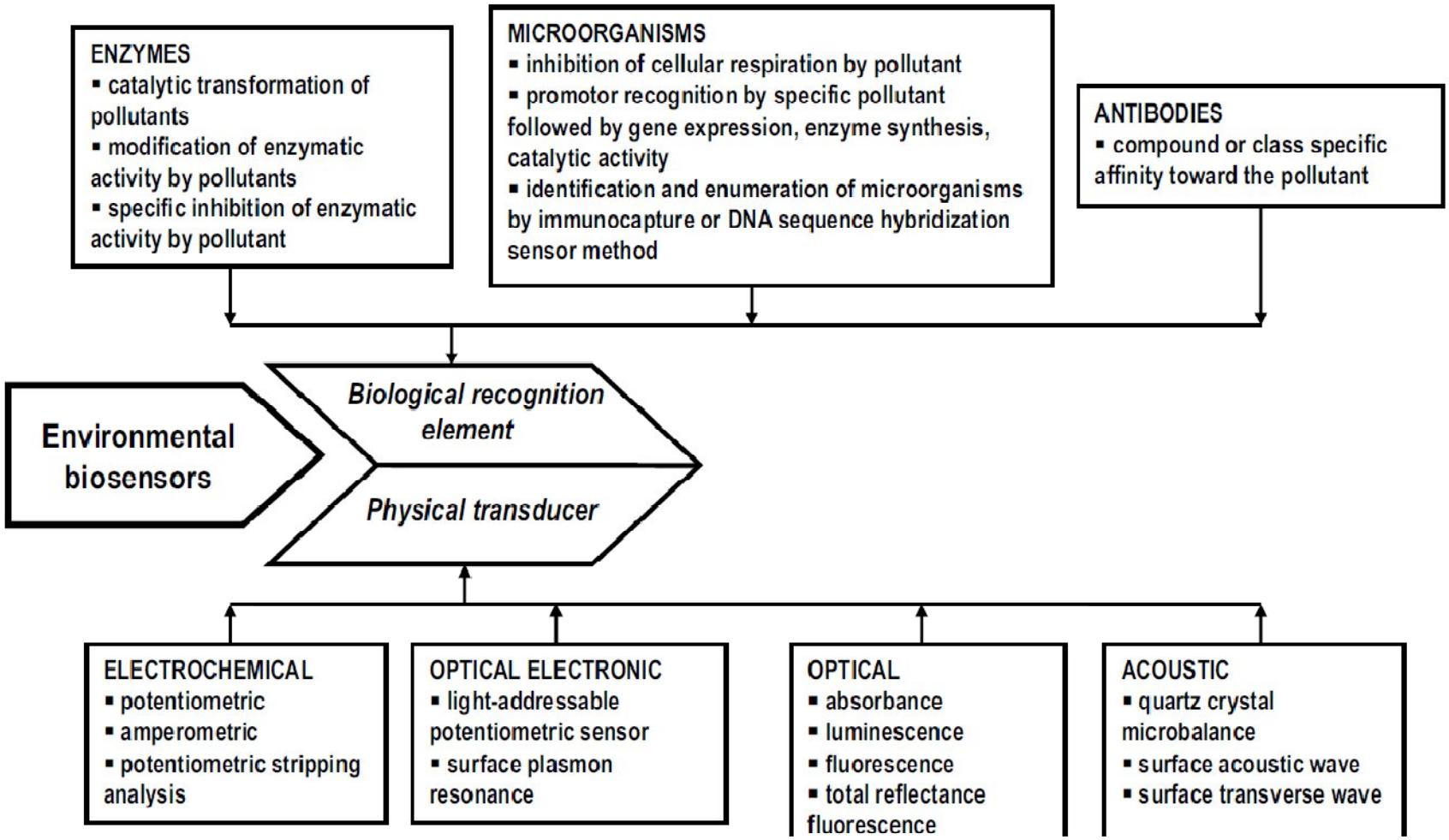
Applications

1. drug delivery systems (DDSs)

2. DNAzyme-based biosensors and nanodevices

3. Treatment the cancer”s and diseases

Applications(11)

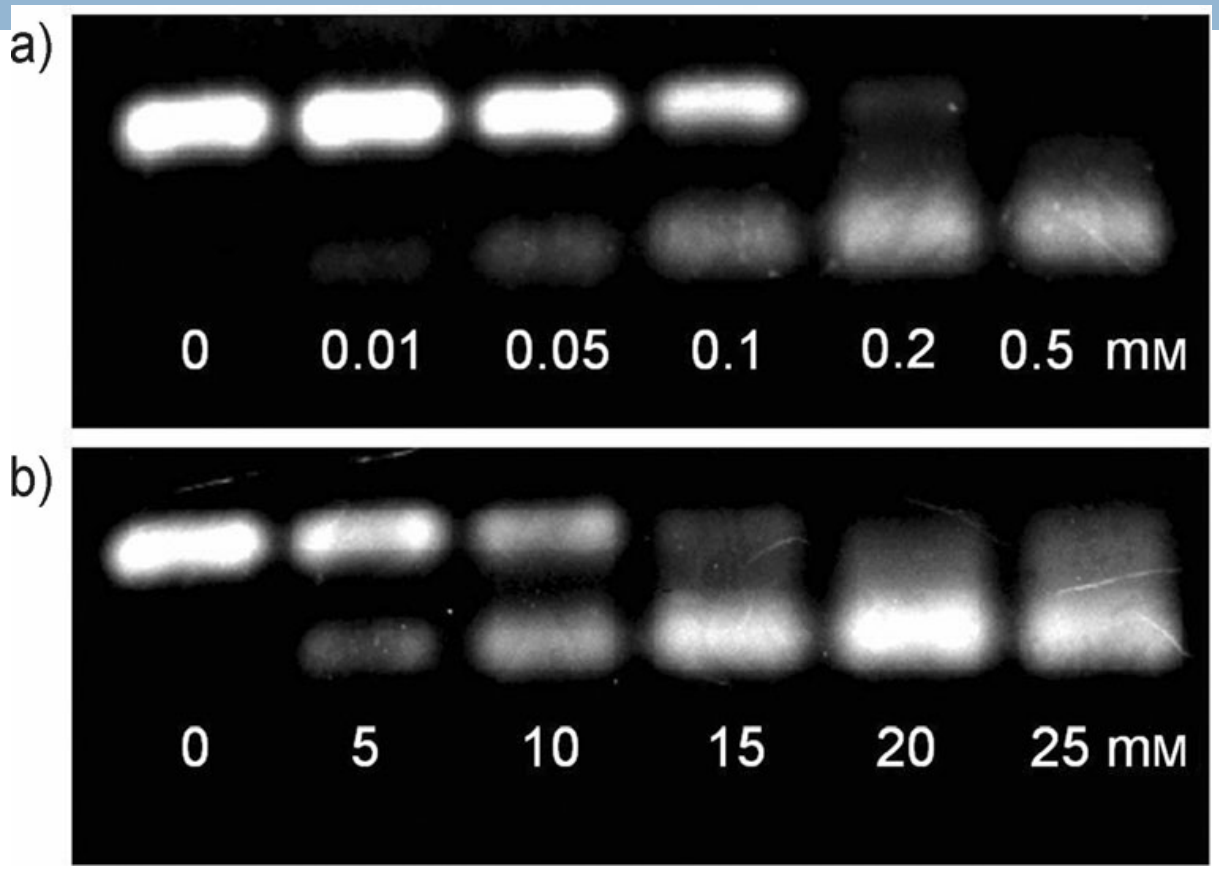


- recognition elements and promising signal amplifiers in biosensor (3)
- based on colorimetry, **Surface Enhanced Raman Scattering**(SERS), fluorescence, or electrochemistry. (3)
- Broad range of cofactors: such as Pb2+,UO2+, Cu2+, Zn2+, and histidine (3)

Different Cofactors(1)

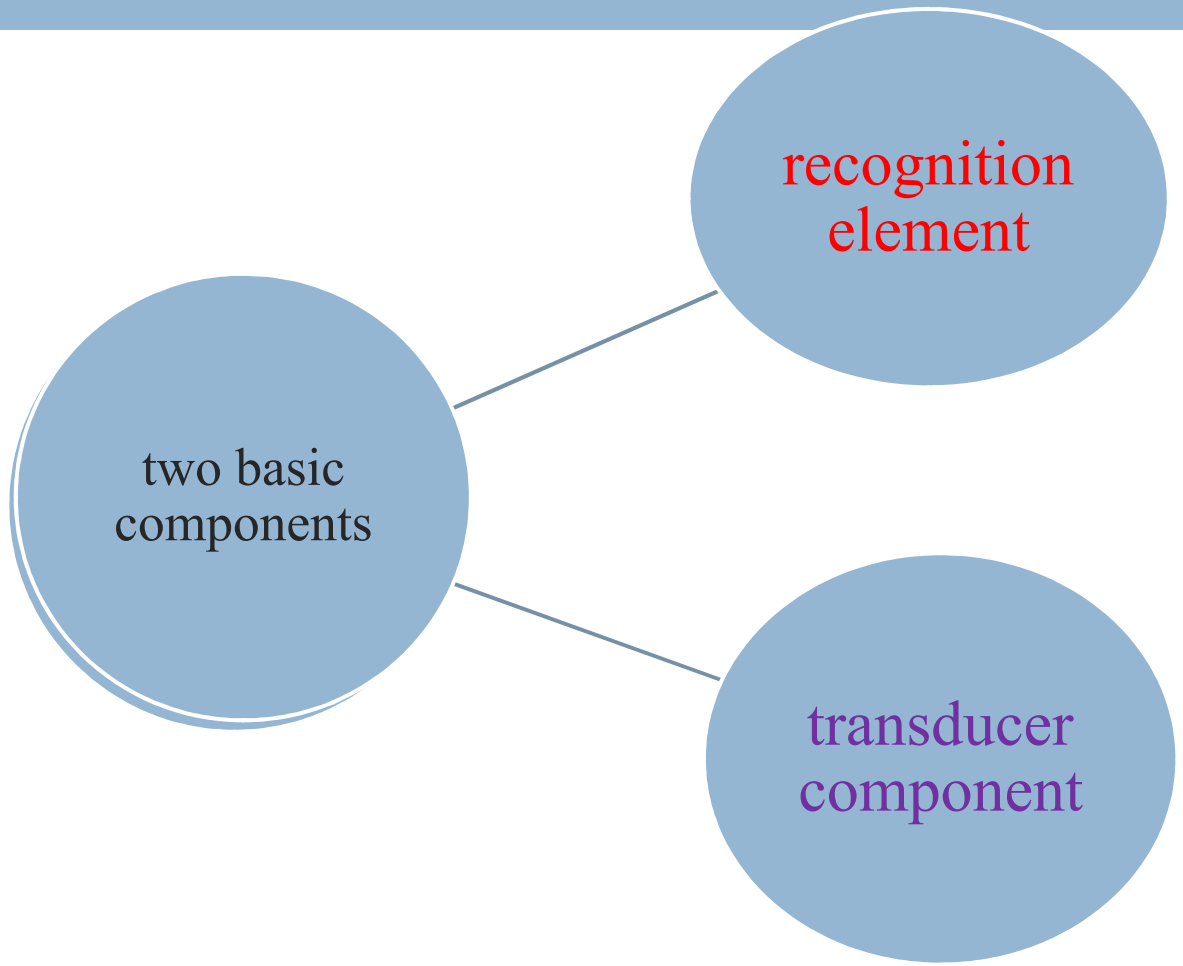
Mn2+

Mg2+



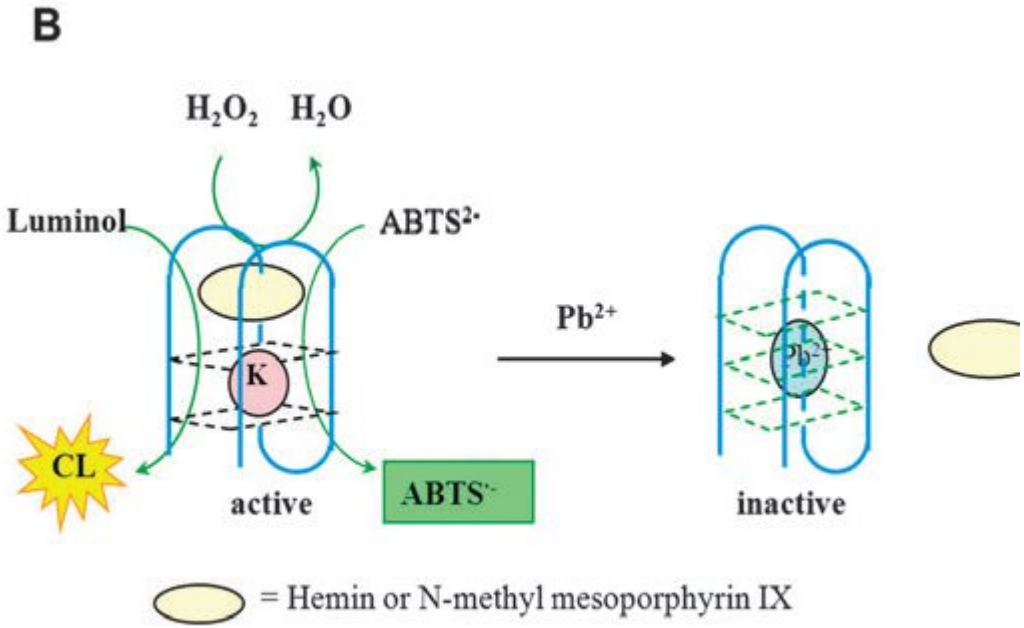
To investigate whether the produced Mn2+ ions from the reduction of MnO2 nanosheets could act as cofactors for 10–23 DNAzyme, the effect of Mn2+ on the catalytic activity of DNAzyme for RNA cleavage was analyzed by agar electrophoresis. It can be seen in Figure 1 that 0.2 mm of Mn2+ ions could trigger 10–23 DNAzyme to completelycleave the substrate within 60 min. However, under the same conditions, a concentration of 15 mm was necessary for Mg2+ ions, indicating that the Mn2+ ion was the more efficientcofactor.

Biosensors

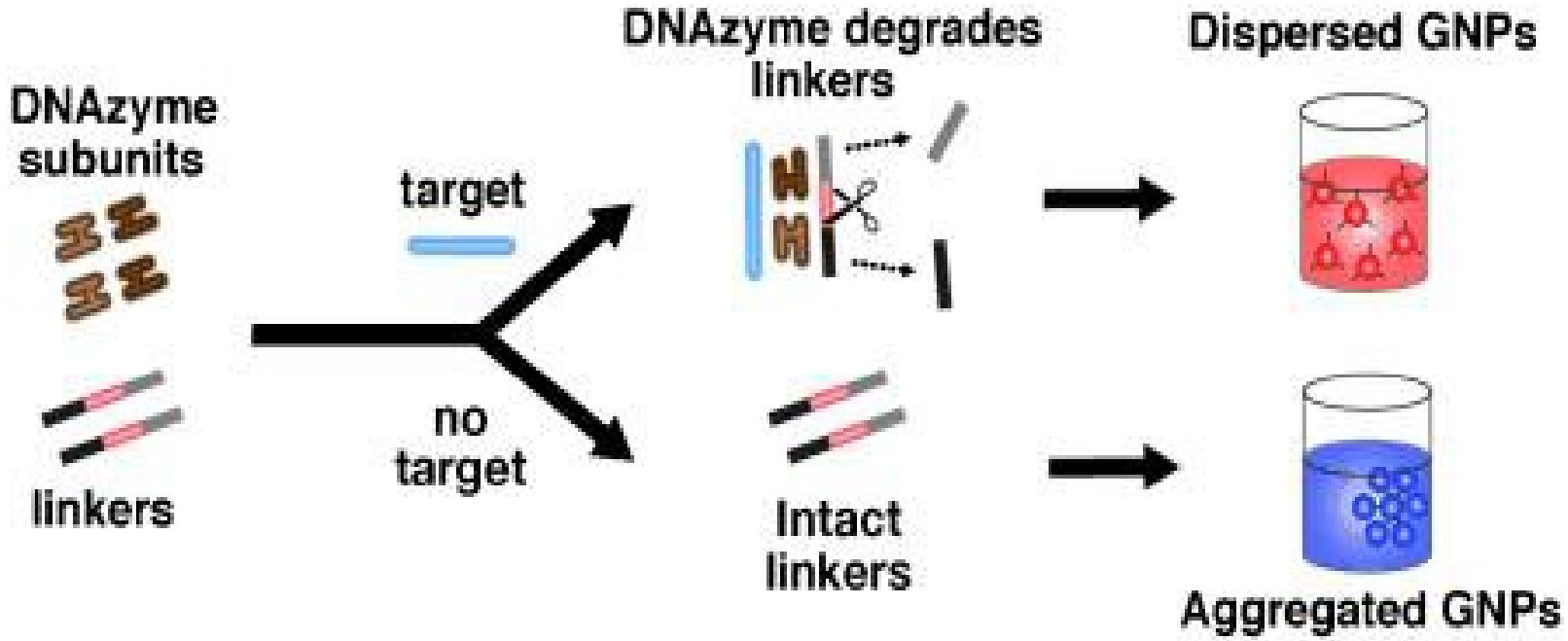

recognition element
two basic components
transducer component

- nanomaterial assisted like AuNPs, GO, CNT, QDs(9)
- provide novel sensing systems based on colorimetry, dynamic light scattering(DLS), or SERS (9)

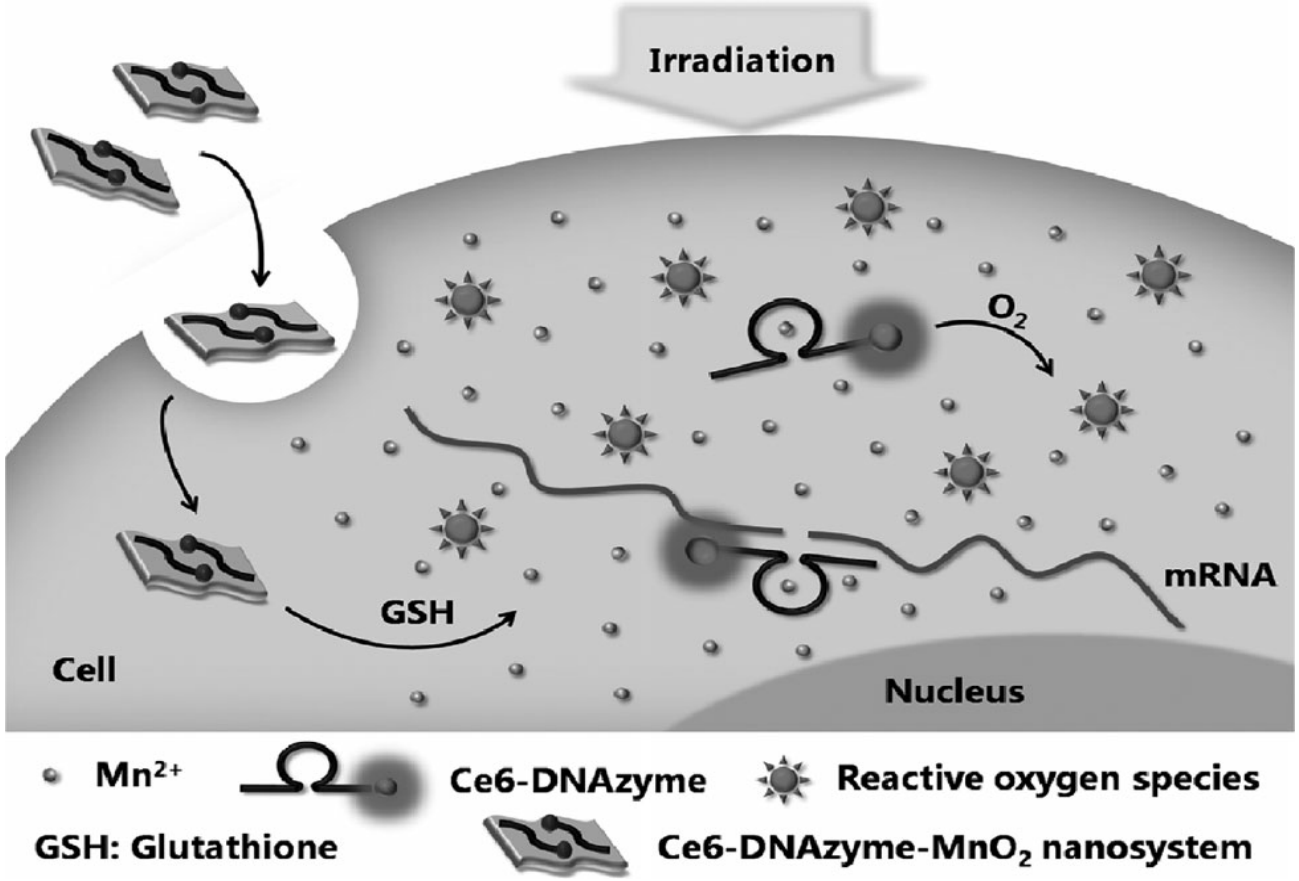
Chemiluminescence (CL)(6)



Colorimetric (7)



Treatment the cancer”s and diseases(1)

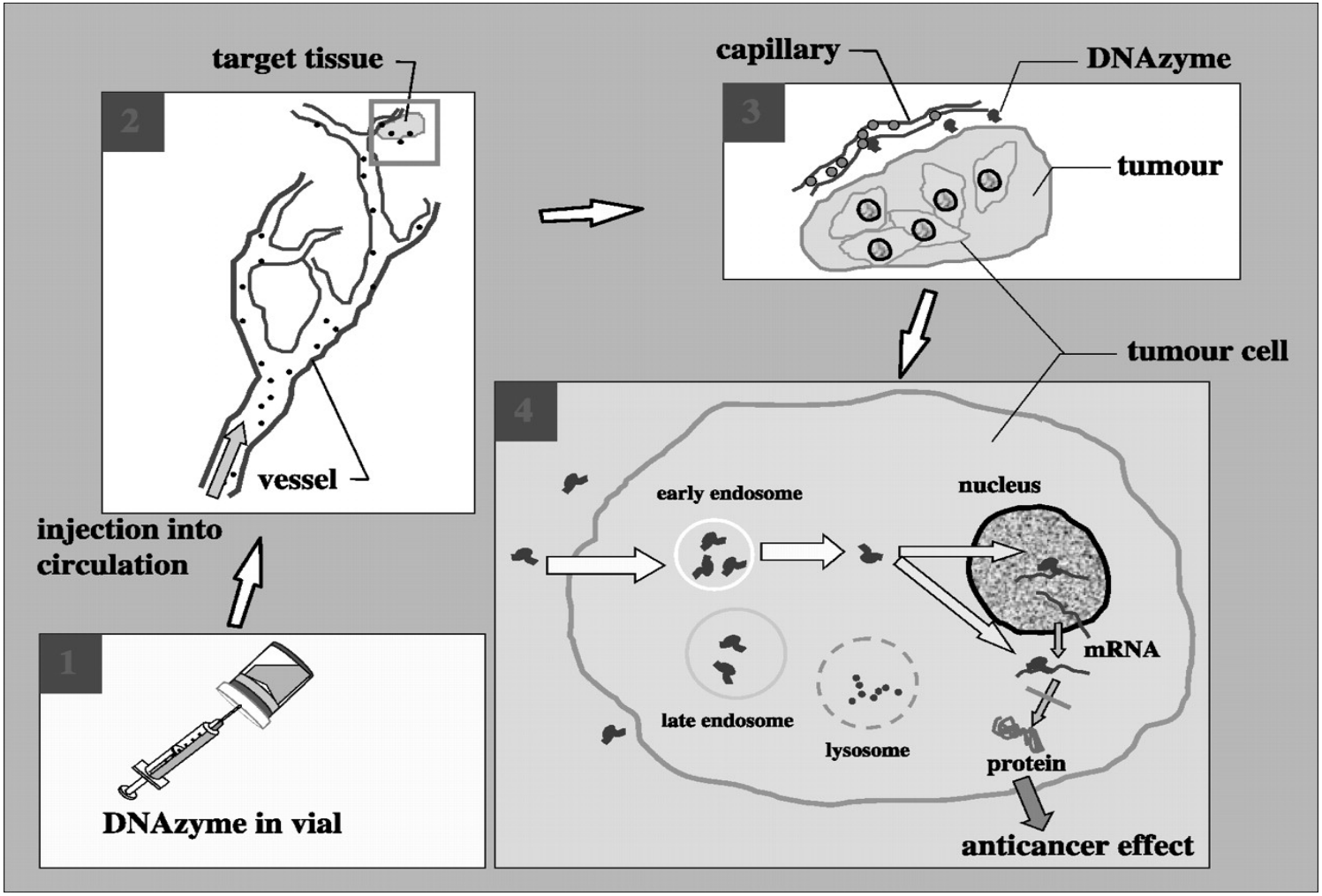


Activity of DNAzymes against Cancer Cells

- inhibited proliferation and induced apoptosis (10)

- induce the release of cytochrome *c* from mitochondria(10)

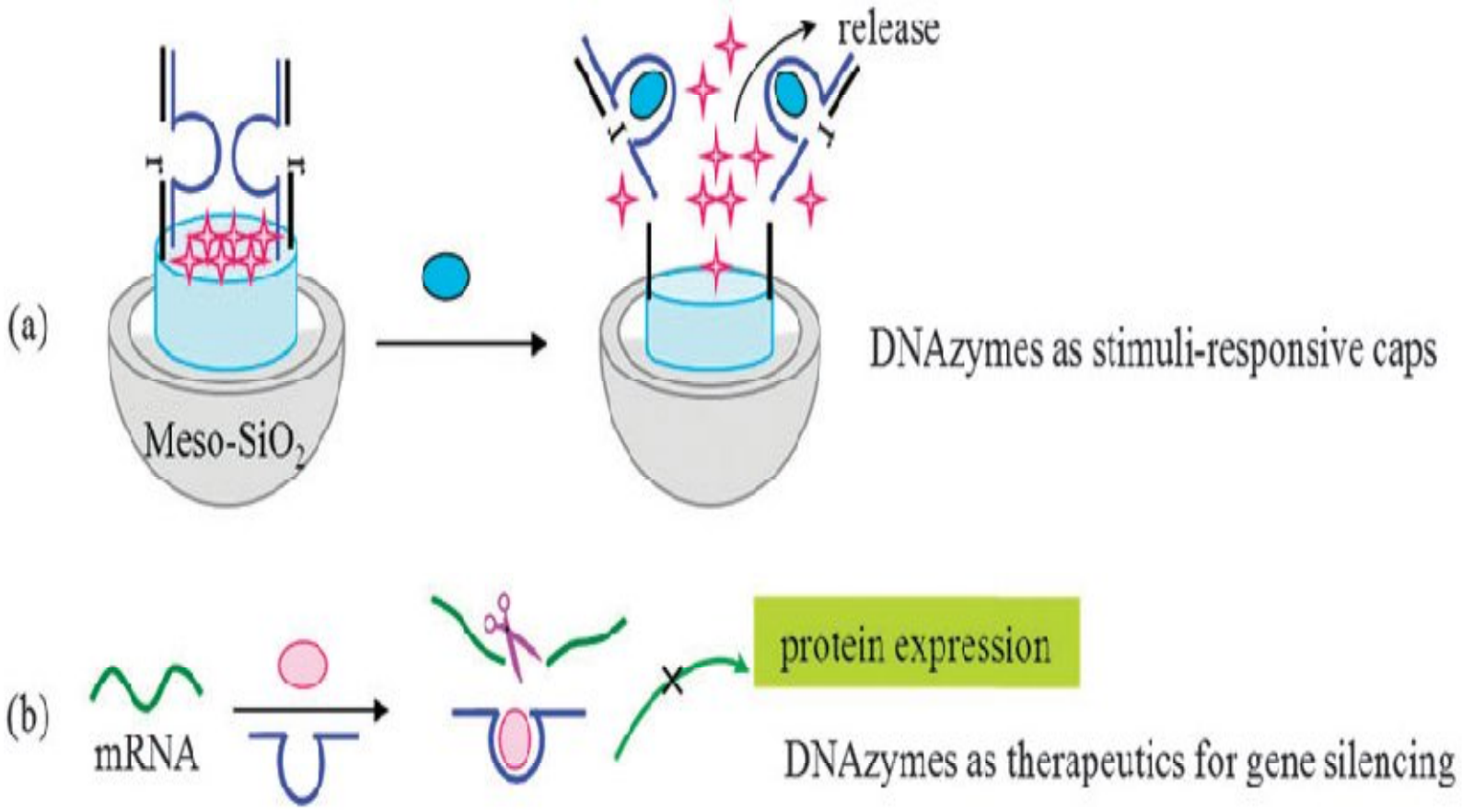
DNAzyme Delivery—Facing the Beast of a Challenge(10)



Drug Delivery Systems (DDSs)

- presenting drug agents against cancer(5)
- target gene knockdown agents(5)
- more emphasis needs to be placed on actual efficacy and safety(5)
- chitosan and polyethylenimine (PEI)(8)

Drug Delivery Systems (DDSs)(3)



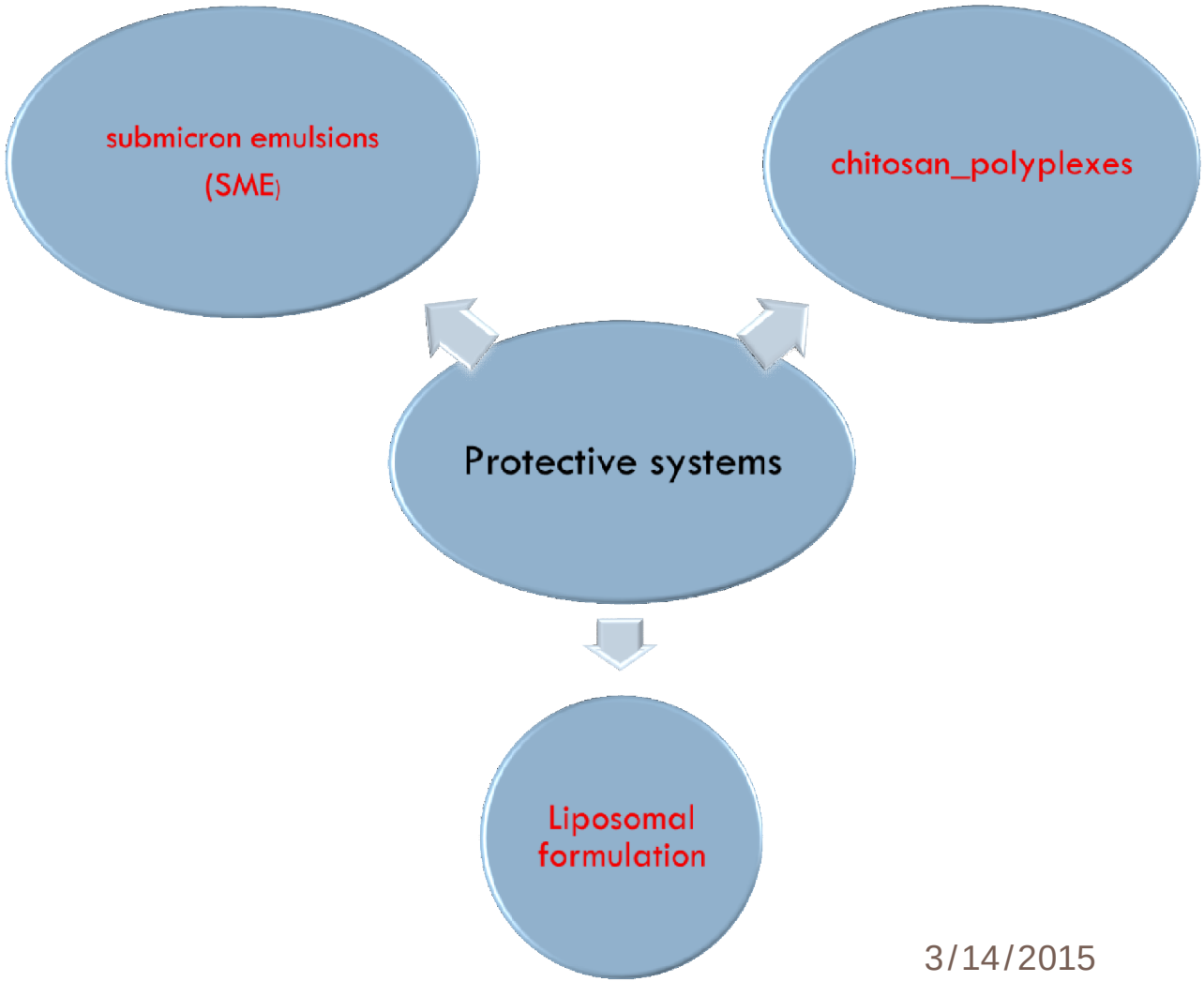
DNAzymes – Short overview of *in vitro* usage.*(5)*

mRNA target	Pathogenesis	DNAzyme usage
Bcr-abl	Chronic myeloid leukaemia (CML), acute lymphoblastic leukaemia (ALL)	Three DNAzymes were designed against two variants of the p210 *bcr-abl gene and p190 variant.* DNAzymes were transfected into CML K562 cells using GS2888 cationic liposomal reagent.
β 1 and β 3 integrin	Important in cell–cell and cell–matrix interactions in the pathogenesis of various tumours.	Two DNAzymes to β 1 and β 3 mRNA were designed to contain a 15-deoxynucleotide catalytic domain fl anked by two substrate recognition segments of 8 and 10 deoxynucleotides for β 1 and β 3 DNAzymes, respectively. DNAzymes were partially modifi ed with phosphorothioate and with 2'-O-methyl groups at both the 5' and 3' ends.
Epidermal growth factor receptor (EGFR)	Receptor tyrosine kinase of the Erb B receptor family that is abnormally activated in epithelial tumours.	DNAzymes targeting the EGFR mRNA.
PML/RAR α fusion gene	Acute promyelocytic leukaemia (APL).	DNAzymes (Dz1 and Dz3) were designed to cleave the PML/RAR α transcript at the GC nucleotides at the fusion point and three nucleotide s upstream of that respectively. Unique quadruple cleavage activity of designed DNAzymes.

DNAzymes – Short overview of *in vivo* usage(5)

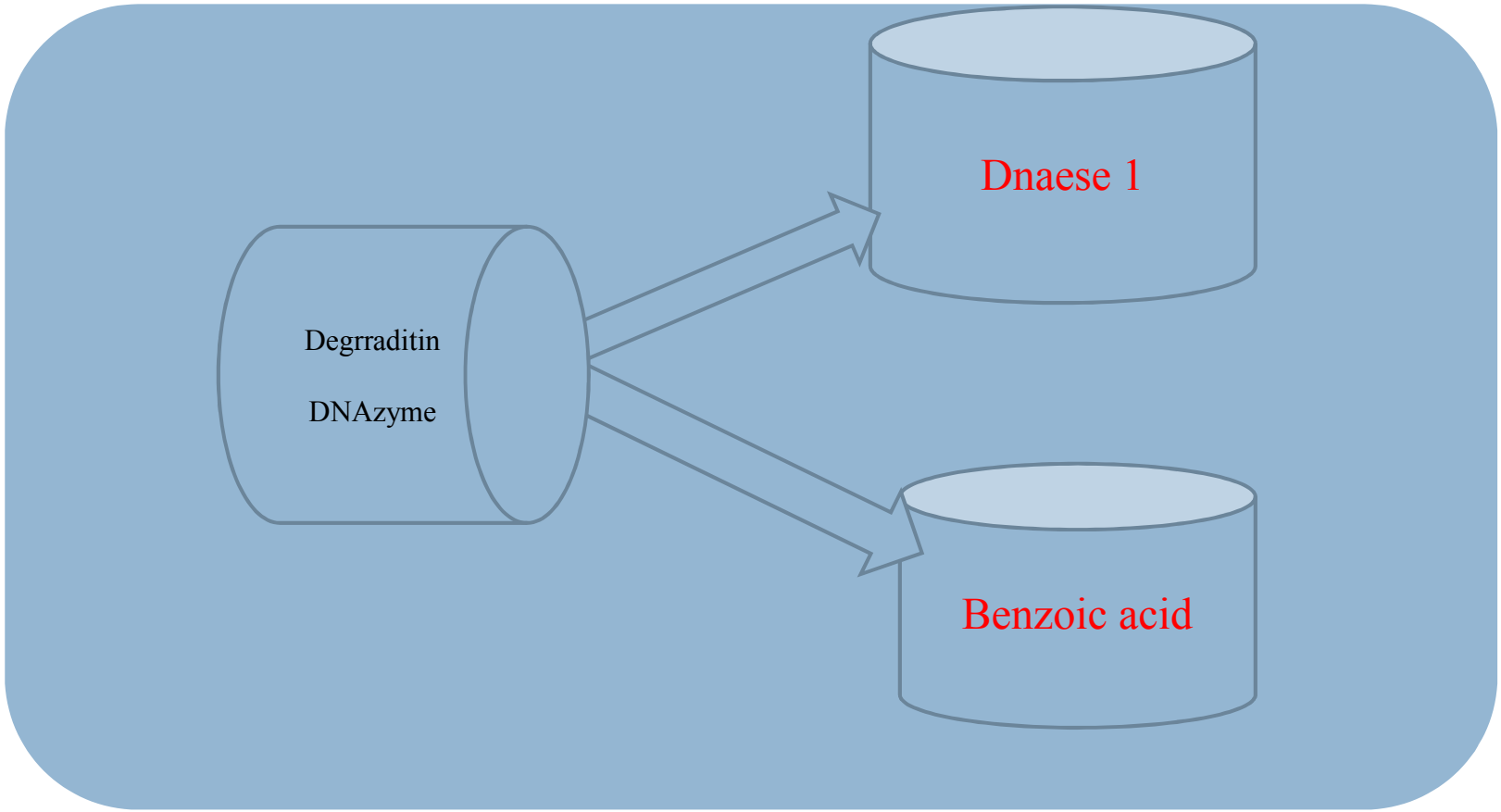
mRNA target	Pathogenesis	DNAzyme usage
Human survivin in PANC-1 cells	Human pancreatic carcinoma – regulates cell division and inhibits apoptosis.	Anti-survivin mRNA DNAzyme designed. Transfected into PANC-1 cells through liposomes.
Urokinase-type plasminogen activator receptor (uPAR)	Implicated in signal transduction and biological processes including cancer metastasis, angiogenesis, cell migration and wound healing.	Three different DNAzymes (Dz372, Dz483, Dz720) targeted against three separate purine and pyrimidine junction in uPAR mRNA.

Discussion (DDS)(8)



Stability of DNAzymes

- pH values from 5 to 7 (2)
- relative humidity (RH) (2)
- Preservatives (2)


Dnaese 1
Degrraditin
DNAzyme
Benzoic acid

DNAzyme recovery (%)

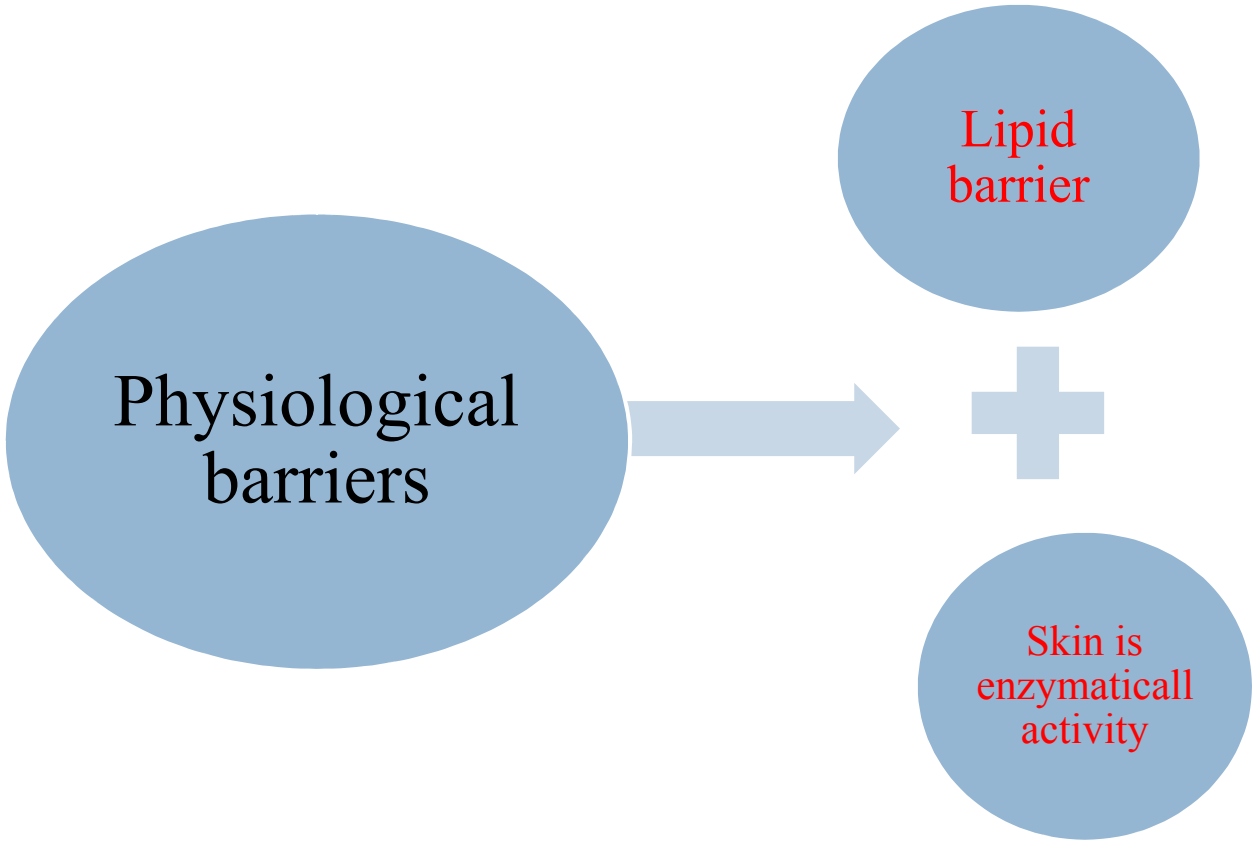
Samples	0.5 h	1.0 h	2.0 h
Aqueous solut.	44	22	7
Acetate buffer	57	30	6
SME	25	10	2
Protective DDS	101	84	70

Dermatitis(Dz13)

- Characteristic(Dz13)

- 10–23 DNAzymes(2)
- Hydrophilic(2)
- high molecular weight (MW) (2)
- negative charge(2)

Dermatitis(Dz13)



Applications the Dz13

- protein c-Jun(2)

- suppressing the growth of squamous and basal cell(2)

Conclusion

- easy to synthesize and functionalize(9)
- multiple enzymatic turnover properties(6)
- high loading efficiency of nanomaterials(4)

Conclusion

- cleave an all-RNA(2)
- mRNA-targeted gene silencing(3)
- gene therapeutic(1)

Conclusion

- hairpins or aptamer–DNAzyme conjugates
- anticipated to appear on the market in the near future
- basic research level

Software

Refrences

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